31

Preliminary Evaluation of Chemicals for Use with Salt in Curing Hides and Skins

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In recent years many chemicals have been suggested for mixing with salt to increase its preservative action in the curing of hides and skins. A sound, small-scale procedure for at least a preliminary evaluation of these chemicals would be very helpful in making for a more critical selection of them. As a result of extended studies over a number of years a laboratory method for this purpose has been evolved which yields, it is believed, informing and valuable results, although it is realized that no such procedure can be accepted as final in place of trial in actual practice. This procedure, together with results obtained by it in the evaluation of many chemicals mixed with salt, is offered for the information of those who may be interested in the subject.

In developing the procedure drastic conditions for the promotion of microbial spoilage were purposely incorporated. Therefore, the test prob-

ably is much more severe than influences ordinarily met with in actual practice. This may mean at times that a chemical, or salting formula, given a comparatively poor rating by the test procedure might make a much better showing in commercial application.

Moisture has been shown to play a critical part in governing microbial activity in salted skins[18,19,20]. The mixing of even small amounts of some materials with salt may markedly affect its hygroscopic properties and those of the hide or skin on which the salt is used. It has been shown[20] that this effect may be great enough with certain materials to bring about pronounced differences in the moisture content of salt-cured hides or skins and that these differences may cause marked changes in the extent of microbial damage that occurs. The moisture content, therefore, should be controlled so that during the test there shall always be present sufficient and comparable amounts of moisture. This can best be done by incubating the pieces of cured skin over water[20]. To accelerate effects, incubation is done at the comparatively warm but not unreasonably high temperature of 30° C.

All hides and skins carry naturally a very heavy and extremely variable inoculum of microorganisms. This is equally true of old salt previously used for curing[14] and of unsterilized solar salt[22]. Conditions for the testing procedure, therefore, should insure a heavy contamination. This is accomplished by using a mixture of equal parts of new G. A. salt; old, bloody, used salt; and crude solar evaporated salt with unwashed hide or skin. The amount of salt is governed by using always a quantity equal to a given percentage of the weight of the skin. This is done not only to effect a uniform and adequate cure but also to regulate the influence of excess salt[18,11].

Since microbial activity is further affected by the salt-soluble proteins[13] that may be present, this factor is controlled roughly by allowing the green-salted test piece of skin to drain or bleed under identical conditions at a given temperature and preventing it from coming in contact with the drainings during the ensuing period of cure or incubation.

Observations have shown that hair-slip and other evidences of microbial activity will develop quicker in the belly and shoulder areas of calfskin than in the back or bend portion. To eliminate this variable, duplicate test pieces of a given area are always taken from the bend location.

The test procedure may be described in detail as follows: A freshly flayed calfskin weighing 8 to 10 pounds is selected and lightly fleshed to remove adhering fat and muscle tissue. The hair is left unclipped. An area corresponding to a double butt bend is cut out and divided into test pieces 3.5 inches square. Each test piece is weighed to 0.1 gram and two of them are selected for each test.

A stock salt is prepared by thoroughly mixing equal quantities of a new G. A. salt, a used salt procured from the hide cellar of a local butcher, and a crude solar evaporated sea salt. This mixture will be referred to subse-

quently throughout the text as the control salt. The chemicals that are to be tested are mixed with the control salt, using for each one a quantity equal to the stated percentage of the weight of the salt.

Over the flesh side of each test piece of calfskin there is spread uniformly a quantity of the salt, with or without added chemicals as the case may be, equal to one-third of the weight of the piece of skin. The duplicate pieces are then placed flesh side up, one on the other, on a glass plate and put in a humid chamber, shown in Figure I.

The glass plate (A, Figure I) is 9.5 cm. square. It is laid in a shallow culture dish (B) having an inside diameter of 15 cm. One edge of the plate is elevated by placing beneath it a glass rod (C) about 1 cm. in diameter.

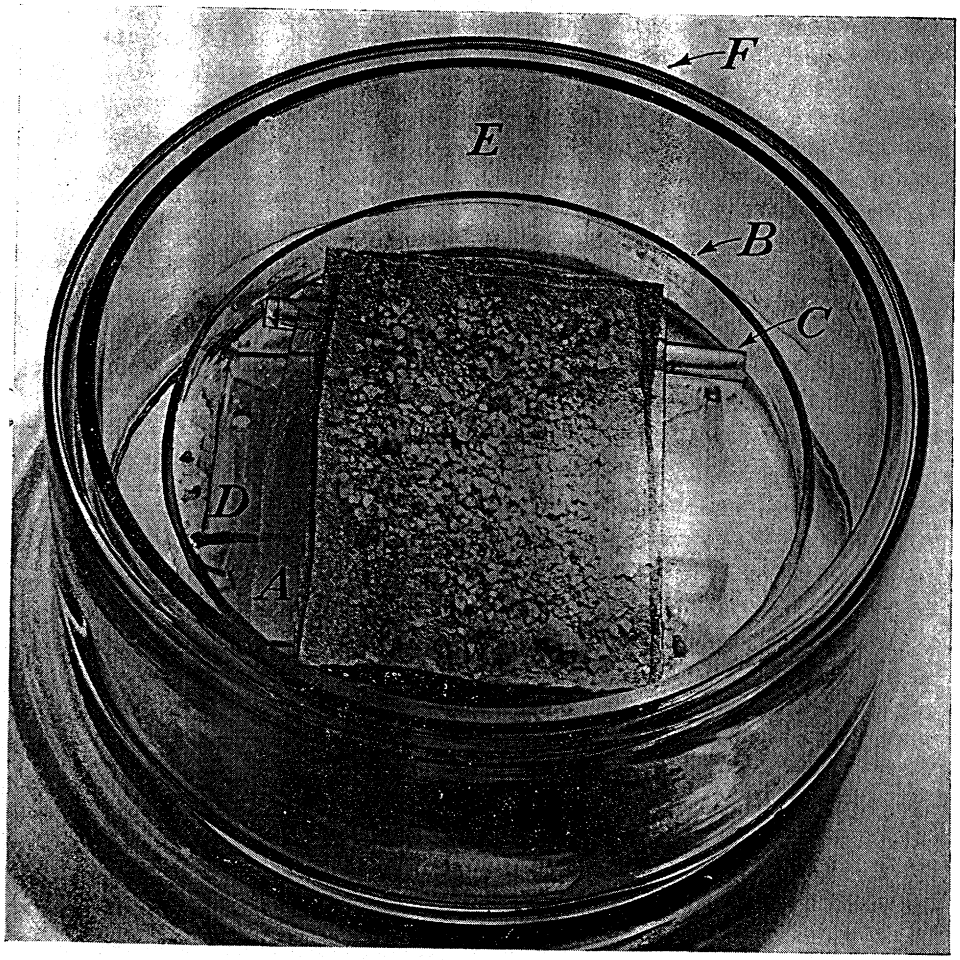

FIGURE I. Humid chamber assembly for small-scale curing tests.

The shallow culture dish rests on a glass platform (D) that is 125 mm. long and 52 mm. wide and stands 20 mm. high. The glass platform is centered on the bottom of a large glass dish (E) having a depth of 70 mm. and an inside diameter of 176 mm. Water is added to this dish to a depth of about 1 cm. The dish (E) is provided with a loose fitting cover (F). The entire assembly is then placed in an incubator and kept at 30° C.

The length of the incubation period has been set at six weeks. This period was chosen after preliminary experiments had shown that pieces of calfskin salted with one-third their weight of control salt, to which no chemicals had been added, invariably showed hair-slip and pronounced reddening on the flesh and had a strong ammoniacal and putrid odor at the conclusion of six weeks' incubation. The pieces, however, seldom displayed any evidence of grain-slip within this time period and thus still could be used as controls in small-scale soaking, liming, bating, pickling, and tanning operations when desired. Many bactericidal substances have a pronounced fixing action on the hair or a tanning effect. This tendency can be noted by carrying the pieces after incubation through the small-scale tanning operations mentioned above and such chemicals thus can be eliminated from further consideration.

The efficiency of preservation may be judged by comparing odor, degree of hair-slip, and appearance of the flesh with similar pieces of skin cured and incubated with the control salt only. Comparisons that are made on this basis, however, do not provide a quantitative evaluation. For this purpose analyses may be made to show changes in bacterial population, total soluble nitrogen and ammonia nitrogen.

In making the chemical analyses and bacterial counts the following procedure has been adopted as quite suitable, bearing in mind ease of manipulation and the comparatively short time required.

The two pieces of calfskin after incubation are removed from the culture dish and soaked at 23° to 28° C. in six times their weight of distilled water for four hours. The weight of water to be added is six times the sum of the original green weight of the unclipped, unwashed skin and the weight of added salt. The pieces are then drained in a funnel for thirty minutes after which they may be discarded or employed for any small-scale tanning operations that have been planned.

The soak water and the drainings that collected in the shallow culture dish during incubation are combined and water is added to make one liter. Aliquots of 50 ml. are taken for the determination of total soluble nitrogen by the K. G. A. method and ammonia nitrogen by magnesium oxide distillation. Three 0.01 ml. aliquots are removed for the direct microscopic counting of bacteria according to the method of Breed, as described in a previous publication[19]. Actually then, the analyses are made on the soak waters and drainings and the results are used as indicative of the state of preserva-

tion of the skin. The nitrogen values obtained in this manner probably do not provide as accurate a picture of the actual changes in the skin proteins as would be the case with a more complete analysis including nitrogen content of the skin itself and volatile nitrogen compounds. However, with a presumptive test of this kind these more refined and time consuming determinations do not seem justified since the additional data thus obtained would not alter appreciably the final results from a comparative viewpoint.

To secure correction factors for the soluble nitrogen products and the bacterial population originally introduced by the contaminated materials used, six tests were made with paired pieces from different calfskins salted with the control salt. For each of these tests there was also used a different lot of control salt made up of G. A. salt, used salt, and solar salt, as previously described but prepared at different times. These pieces were incubated for only ninety-six hours, which was considered to be a period short enough to avoid appreciable bacterial multiplication but long enough to permit thorough penetration of the salt into the skin and the relatively complete draining away of the salt-soluble proteins. The results of these tests are given in Table I.

TABLE I

Total soluble-nitrogen content, ammonia-nitrogen content, and bacterial population, using calfskin cured with control salt and incubated over water at 30° C. for 96 hours.

	Results expressed on basis of 1 gram of unwashed, unclipped, freshly flayed calfskin.		
Test No.	Total soluble nitrogen equivalent mls. 0.1*N* HCl	Ammonia nitrogen equivalent mls. 0.1*N* HCl	Bacterial count millions
1	0.166	0.136	57
2	0.215	0.127	120
3	0.183	0.081	177
4	0.188	0.115	163
5	0.203	0.124	215
6	0.178	0.092	86
Average	0.189	0.113	136

In view of the highly contaminated nature of skins and used salt, the results in Table I may be considered as quite consistent and, as will be shown later, are of a low order of magnitude when compared with corresponding data from pieces of skin incubated for six weeks with control salt only.

The average values in Table I for total soluble nitrogen and ammonia nitrogen, respectively, have been subtracted in each case from those obtained after six weeks incubation. In other words, only the increase in these nitrogen values resulting from six weeks' incubation, instead of ninety-six hours, is shown. The bacterial count from the six weeks' incubation has been stated as a multiple of the average shown in Table I for ninety-six hours' incubation.

The expression of the results in this manner would appear logical, as

indicating the microbial activity that occurred during the prolonged in-incubation. Any error due to the use of average results in place of the respective blank for each lot of control salt and skin may be considered as negligible.

The results obtained after six weeks' incubation over water at 30° C. by the procedure just described with a great many chemicals mixed with salt are given in Table II. It is believed that these results represent a fairly true picture of comparative preservative efficiency as regards microbial spoilage. They do not necessarily reflect the influence of the chemicals themselves upon the structure and strength of the hide or skin, or the properties, dependent thereon, of the leather that might be made from them. In some cases, as will be pointed out, there are suggestions of hydrolysis of the skin from a chemical cause, such perhaps as too great an acidity, which might lead to a weakening of the strength of the resulting leather.

At the risk of repetition but for the sake of clarity, attention is again called to the fact that the percentage figures given with the chemicals in Table II are based on the weight of the salt. For example, sodium silicofluoride, 1 per cent, plus paranitrophenol, 0.1 per cent, means that one part by weight of the former and 0.1 part by weight of the latter were mixed thoroughly with one hundred parts by weight of the control salt.

The pieces of skin from the tests listed in Table II were soaked and unhaired in a lime suspension. None showed evidence of the fixation of hair, from which it may be concluded that the various chemicals, at least in the concentrations used, were satisfactory in not showing a pretannage effect.

In this table the ratio of the ammonia nitrogen to the total soluble nitrogen has been given. In Table I this ratio appears to be quite variable. However, with the incubated samples where a number of tests were made this ratio was found to be rather constant and characteristic for each test. Variations in this ratio may be indicative of effects of the added chemicals on bacterial growth and protein digestion not clearly indicated by the individual values for ammonia nitrogen and total soluble nitrogen.

When the control salt only was used, marked increases in bacterial count, total soluble nitrogen, and ammonia nitrogen were found. The amount of salt applied was sufficient to obtain uniform distribution over the flesh surface of the test pieces and to maintain a slight excess of salt crystals for the six weeks' incubation period, in spite of the constant leaching away of salt by the moisture taken up. Under commercial conditions, Hausam[10] reports 30 per cent salt as the smallest amount that can be used for complete coverage of the flesh. Since the test pieces were taken from the thicker, more compact area of the skin, the quantity of salt used, equivalent to one-third of the weight of the skin, would be comparable to around 40 per cent salt on the basis of a whole skin, which, according to Hausam[10], is

TABLE II

The Comparative Curing Efficiency of Salt Plus Various Chemicals

Chemical and Amount Added to Control Salt	Number of Tests	Appearance of Test Pieces after Six Weeks' Incubation over Water at 30° C.	Increase in Total Soluble Nitrogen over Control[1] ml. 0.1N HCl	Increase in Ammonia Nitrogen over Control[1] ml. 0.1N HCl	Ratio of Ammonia Nitrogen to Total Soluble Nitrogen	Bacterial Count as Multiple of Control[1]
Control salt only	6	flesh red, slimy; hair slip; putrid and ammoniacal odor	1.348	0.892	0.67	28.9X
Sodium silicofluoride, 1% + paranitrophenol, 0.1%	5	flesh slightly yellow; no hair slip; no bad odor	0.356	0.014	0.04	1.2X
Sodium silicofluoride, 1% + sodium pentachlorphenate, 0.1%	1	flesh clean, white; no hair slip; slight phenolic odor	0.312	0.000	0.00	1.2X
Sodium silicofluoride, 1% + sodium (2, 4, 5) trichlorphenate, 0.1%	1	flesh clean, white; no hair slip; slight phenolic odor	0.394	0.023	0.06	1.6X
Sodium silicofluoride, 1%	1	flesh clean, white; no hair slip; musty odor	0.433	0.087	0.20	2.4X
Sodium fluoride, 1%	1	flesh pink; no hair slip; no bad odor	0.747	0.359	0.46	6.6X
Acid sodium fluoride, 1%	1	flesh red, in spots; no hair slip; no bad odor	0.666	0.321	0.48	9.9X
Sodium (2, 4, 5) trichlorphenate, 0.2%	2	flesh slightly dingy; no hair slip; sweet phenolic odor	0.349	0.026	0.08	2.5X
Sodium (2, 4, 6) trichlorphenate, 0.2%	2	flesh slightly dingy; no hair slip; sweet phenolic odor	0.636	0.167	0.24	4.6X
Sodium pentachlorphenate, 0.2%	2	flesh yellow, red in streaks; no hair slip; fishy odor	1.595	0.723	0.45	11.9X
Sodium pentachlorphenate, 0.5%	2	flesh dingy; no hair slip; musty odor	0.759	0.770	1.0	7.9X
Sodium sulfite (anhydrous), 1.0%	2	flesh pink, slimy; sweat slip of hair; putrid odor	1.699	1.690	1.0	23.2X
Sodium sulfite (anhydrous), 1.5%	4	flesh with few red spots; no hair slip; fishy odor	0.438	0.218	0.47	4.8X
Sodium sulfite (technical-anhydrous), 1.5%	2	flesh with brown iron stains; no hair slip; slight fishy odor	0.333	0.335	1.0	4.1X
Acid sodium sulfite, 1.5%	6	flesh red, slimy; hair slip; putrid odor	2.233	1.525	0.67	28.3X
Acid sodium sulfate, 1.5%	2	flesh red, slimy; hair slip; putrid odor	1.330	1.088	0.81	21.7X
Sodium sulfate (anhydrous), 40% (synthetic Khari salt)	1	flesh red, slimy; hair slip; putrid odor	1.741	0.528	0.30	29.6X
Sodium carbonate (anhydrous), 3%	2	flesh red; some hair slip; strong ammoniacal odor	1.026	0.429	0.41	23.1X
Sodium carbonate (anhydrous), 1.0% + sodium pentachlorphenate, 0.1%	1	flesh bright red; slight trace hair slip; slight ammoniacal odor	2.331	2.117	0.90	17.6X
Sodium carbonate (anhydrous), 1.0% + sodium (2, 4, 5) trichlorphenate, 0.1%	1	flesh red, slimy; slight trace hair slip; mousey odor	1.188	0.291	0.22	10.1X
Sodium carbonate (anhydrous), 1.0% + naphthalene, 1.0%	2	few red spots on flesh; no hair slip; naphthalene odor	0.743	0.189	0.25	13.2X

Sodium perborate, 2.0%	1	flesh red; no hair slip; ammoniacal odor	0.909	0.284	0.31	11.2X
Potassium perchlorate, 2.0%	2	flesh red in spots; no hair slip; ammoniacal odor	1.032	0.420	0.41	10.9X
Zinc chloride, 0.5%	1	flesh red; no hair slip; slight ammoniacal odor	0.735	0.145	0.19	17.6X
Zinc oxide (commercial grade), 1.3%	2	flesh yellow, slimy; trace of hair slip; putrid odor	1.277	0.815	0.64	16.9X
Zinc oxide (colloidal), 1.3%	2	flesh yellow, slimy; trace of hair slip; slight ammoniacal odor	0.517	0.336	0.65	8.5X
Boric acid, 2.0%	1	flesh traced with pink; no hair slip; odor not bad	0.742	0.334	0.45	11.4X
Beta naphthol, 0.2%	1	flesh reddened; hair slip; offensive odor	1.062	0.653	0.61	22.6X
Beta naphthol, 0.5%	1	flesh clean; no hair slip; odor fresh	0.461	0.147	0.32	5.4X
Orthodichlorbenzene, 0.2%	1	flesh red; hair slip; odor sweet	1.270	0.609	0.48	34.6X
Phenothiazine, 0.4%	2	flesh with red and blue stains; hair slip; offensive odor	0.747	0.485	0.67	14.0X
N-N′-dichloroazodicarbonamidine-bicarbonate mixture, 0.02%[2]	2	flesh red in spots; some hair slip; slight putrefactive odor	1.133	0.443	0.39	9.5X
N-N′-dichloroazodicarbonamidine-bicarbonate mixture, 0.2%[2]	4	flesh red in spots; no hair slip; no bad odor	0.463	0.157	0.34	7.3X
N-N′-dichloroazodicarbonamidine-bicarbonate mixture, 0.5%[2]	2	flesh red in spots; no hair slip; no bad odor	0.482	0.454	0.94	8.7X
N-N′-dichloroazodicarbonamidine-bicarbonate mixture, 1.0%[2]	1	flesh red in spots; no hair slip; no bad odor	0.520	0.191	0.37	5.8X
N-N′-dichloroazodicarbonamidine-phosphate mixture, 0.2%[3]	2	flesh red and slimy; hair and grain slip; offensive odor	1.694	1.600	0.93	29.2X
Sodium acetate-monochloracetic acid-potassium bromide mixture[4]	2	flesh with white mold; no hair slip; musty, fishy odor	0.684	0.570	0.83	6.6X
Sodium acetate-monochloroacetic acid-potassium bromide mixture[4] + paranitrophenol, 0.1%	2	flesh clean; no hair slip; no offensive odor	0.427	0.029	0.07	4.3X
Acetate buffer[5], pH 5.6, 1.0% + paranitrophenol, 0.1%	1	flesh red; no hair slip; no offensive odor	1.029	0.616	0.59	14.4X
Acetate buffer[5], pH 5.6, 1.0% + sodium pentachlorphenate, 0.1%	1	flesh red; no hair slip; mousey odor	1.284	0.596	0.46	9.3X
Acetate buffer[5], pH 5.6, 1.0% + sodium (2, 4, 5) trichlorphenate, 0.1%	1	flesh pink; no hair slip; no offensive odor	0.566	0.305	0.54	8.0X

[1]Expressed on basis of 1 gram of unwashed, unclipped, freshly flayed skin.

[2]Commercial mixture of 33 per cent N-N′-dichloroazodicarbonamidine in sodium bicarbonate.

[3]Commercial mixture of 1.14 parts N-N′-dichloroazodicarbonamidine to 2.27 parts disodium phosphate, 0.34 parts monopotassium phosphate and 32.18 parts sodium chloride.

[4]0.5 per cent sodium acetate, 0.2 per cent monochloracetic acid, and 1.0 per cent potassium bromide added.

[5]Acetate buffer, pH 5.6, prepared by mixing 1 mol. of acetic acid with 8 mols. of sodium acetate.

about the correct amount of salt. The quantity of salt used can be considered, therefore, as adequate for effective curing.

Where 1 per cent sodium silicofluoride was used in combination with 0.1 per cent paranitrophenol, sodium trichlorphenate or sodium pentachlorphenate, the increases in bacterial count and ammonia nitrogen were negligible. There was a slight but definite increase in total soluble nitrogen that may have been due to acid hydrolysis, since the ratio of ammonia nitrogen to total soluble nitrogen was very small. Sodium silicofluoride alone, in a concentration of 1 per cent, was quite effective as a preservative but the values for total soluble nitrogen and ammonia nitrogen were slightly higher than in those tests where it was used with the three above-named chemicals. The ratio of the ammonia nitrogen to total soluble nitrogen was three to four times as high, and the bacterial count was twice as great. These results confirm previous reports[5,21] on the effectiveness of sodium silicofluoride when used with a fungistatic material. Whether or not acid hydrolysis, as indicated by the increase in total soluble nitrogen, is great enough to bring about adverse effects in finished leathers made from hides or skins cured with sodium silicofluoride is a question that can only be determined by commercial salting and tanning tests. Some information on this aspect is being acquired from experiments under way.

Sodium fluoride and acid sodium fluoride both increased the preservative efficiency of salt when added to the extent of 1 per cent. Apparently both compounds give about the same degree of preservation. The increases in total soluble nitrogen, ammonia nitrogen, and the bacterial count are considerably greater than was observed with an equal concentration of sodium silicofluoride. The ratio of ammonia nitrogen to total soluble nitrogen is more than twice as great.

With regard to acid sodium fluoride, it should be brought out that this is not a true salt but a mixture which may be represented by $(NaF)_x.(HF)_y$. Thus, the results obtained with a particular product might be at variance with those obtained with another product sold under a similar label. The solubility of the product varies considerably with the NaF:HF ratio[17].

Sodium (2, 4, 5) trichlorphenate in a concentration of 0.2 per cent gave excellent preservation. There was only about a twofold multiplication in the bacterial count and the increases in total soluble nitrogen and ammonia nitrogen were very small. The ratio of ammonia nitrogen to total soluble nitrogen was low, indicating that bacterial decomposition of the soluble proteins had been very slight.

Sodium (2, 4, 6) trichlorphenate, on the basis of the appearance of the test pieces, seemed to be as effective as the salt of the 2,4,5 derivative. According to the analytical data, however, it was not quite so. The increases in bacterial count and total soluble nitrogen were about twice as great as with the 2,4,5 product and the ratio of ammonia nitrogen to total soluble

nitrogen was three times as high. From a practical standpoint, however, the 2,4,6 compound would be classed as an effective preservative.

The results with the sodium salts of the trichlorphenols confirm earlier reports[5,21] with regard to their effectiveness as preservatives when used in low concentrations with salt in curing animal skins.

Sodium pentachlorphenate, even in a concentration of 0.5 per cent, was not as efficient as either of the trichlorphenate salts. At a concentration of 0.2 per cent the increases in total soluble nitrogen and ammonia nitrogen were approximately of the same magnitude as with the control salt alone, although the bacterial count was not nearly so great. With 0.5 per cent the increase in total soluble nitrogen was only about one-half as great, but the increases in the ammonia nitrogen and bacterial count were not materially different than with the lower concentration. The overall effect of sodium pentachlorphenate on the bacterial cells is definitely not great enough to completely suppress growth in the concentrations studied although it may limit to some extent the digestive processes of bacteria. Small-scale tests indicated that this compound might be slightly safer from the standpoint of protein fixation than the sodium salts of trichlorphenate in higher concentrations. Hausam[10] has reported that trichlorphenol has a hardening influence on skins when used in high concentrations. Further studies will be necessary, however, to establish exactly the maximum concentrations of the various chlorinated phenols that can be used with safety in hide curing.

Anhydrous sodium sulfite in a concentration of 1.5 per cent gave what might be called excellent preservation. The appearance of the pieces was good and the bacterial count was low. The increases in total soluble-nitrogen and ammonia-nitrogen values were also very low. At a concentration of 1 per cent, however, this chemical was relatively ineffective and the high values for total soluble nitrogen and ammonia nitrogen, together with the fact that the ratio of ammonia nitrogen to total soluble nitrogen increased to 1, would indicate that at this concentration hide-protein degradation had been stimulated. The bacterial count was approximately the same as that found with the control salt. The permanence of preservation with sodium sulfite remains to be determined since the sulfite would tend to be oxidized to sulfate, depending upon pH and other factors. A practical objection to anhydrous sodium sulfite is that technical grades carry traces of iron which resulted in staining in these tests. Thus, commercially, it would be necessary to use the iron-free, hydrated, technical sodium sulfite in concentrations equivalent to the anhydrous salt.

Acid sodium sulfite[15] in a concentration of 1.5 per cent appears to stimulate protein degradation by bacteria.

Although the bacterial count was of the same order as that obtained with the control salt, the increases in the total soluble nitrogen and ammonia nitrogen were much greater. This would tend to lend credence to the claim

of Babakina and Kutukowa[1] that acid sodium sulfite added to salt has a harmful influence in the preservation of salted hides and skins.

Acid sodium sulfate in a concentration of 1.5 per cent, as recommended by Robertson[15], did not increase the preservative efficiency of salt as judged either by appearance or by the analytical results.

A synthetic "Khari" salt as recommended by Das, Davahle, and Pal[4], or a salt made up with the control salt mixture to contain 40 per cent by weight of anhydrous sodium sulfate, did not show any greater preservative efficiency than salt alone as reflected by increases in total soluble nitrogen, ammonia nitrogen, and bacterial count. The ratio of ammonia nitrogen to total soluble nitrogen was considerably lower than with the control salt, being about 0.3. Thus, it is possible that the sodium sulfate exerts some type of selective or restraining influence upon the protein metabolism of the bacteria and that this would show up in a beneficial manner in the quality of finished leather made from skins cured with "Khari" salt. This possibility can only be considered as hypothetical and much work would be necessary to establish it.

Three per cent of anhydrous sodium carbonate did not show satisfactory preservation. On the basis of appearance the pieces were as badly decomposed as those cured with the control salt. However, when rated by bacterial count, total soluble nitrogen, and ammonia nitrogen, a slight preservative action might be ascribed to soda-salt. It should be pointed out here that Bergmann and Seligsberger[3] recognized the poor bacteriostatic action of soda but prescribed its use for the neutralization of such impurities as the acid salts of calcium and magnesium, commonly found in commercial salt.

When added with 1 per cent of sodium carbonate, 0.1 per cent of sodium pentachlorphenate or 0.1 per cent of sodium trichlorphenate did not give satisfactory preservation. The sodium trichlorphenate mixture was more effective than the sodium pentachlorphenate mixture as judged by the increases in bacterial count, total soluble nitrogen, and ammonia nitrogen. However, neither mixture was as effective as 1 per cent anhydrous sodium carbonate plus 1 per cent naphthalene. This mixture markedly increased the preservative action of salt as judged by appearance and lower values for total soluble nitrogen and ammonia nitrogen. The increases in these values, however, were twice as high as with the treatments in which 1 per cent of sodium silicofluoride had been used, and the bacterial counts were from six to thirteen times as high. Nevertheless, on the whole the results can be looked upon as substantial confirmation of the effectiveness of sodium carbonate and naphthalene as found by Bergmann[2] and Hausam[10].

Both sodium perborate and potassium perchlorate at a concentration of 2 per cent, definitely increased the preservative efficiency of salt. These materials were considered as promising, inasmuch as perchlorates and

perborates are strong oxidizing agents and from the standpoint of the production of a high oxidation-reduction potential, or Eh value, should be inhibitory to bacterial multiplication in the presence of high concentrations of salt[23]. Judged by the increase in total soluble nitrogen, neither salt could be said to be very effective. However, the values for ammonia nitrogen and bacteria count were much lower than with the control salt.

Zinc chloride added at a concentration of 0.5 per cent definitely increased the preservative efficiency of the control salt. Zinc chloride, however, is quite acid and the low ratio of ammonia nitrogen to total soluble nitrogen of 0.19, found with the test pieces, may indicate pronounced acid hydrolysis. Guthrie and Sastry[9] found that zinc chloride might cause a "bubbled grain" in finished leathers and this, if so, would rule against its use.

The nonacidic nature of zinc oxide may make it more desirable for use with hides and skins than zinc chloride. Zinc oxide when added to salt at a concentration of 1.3 per cent as recommended by Grassman and Hausam[7,8] produced some, but not marked, improvement in preservative efficiency, as indicated by values for total soluble nitrogen, ammonia nitrogen, and bacterial count lower than those found with the control salt. Colloidal zinc oxide at the same concentration was much more effective as a preservative than ordinary commercial zinc oxide. The correlation of increased preservation with decrease in particle size indicates that the bacteriostatic activity of zinc oxide is associated with its chemical activity and not to an oligodynamic action as suggested by Hausam[10].

Boric acid in a concentration of 2 per cent increased the preservative efficiency of salt appreciably as shown by both general appearance and the analytical results.

Beta-naphthol in a concentration of 0.2 per cent was not an efficient preservative but at a concentration of 0.5 per cent very good preservation was effected, which bears out certain claims made in U. S. Pat. No. 196170[24] as to the possible use of naphthols with salt in curing hides and skins. Concentration seems to be an important factor, as with an increase in concentration of from 0.2 to 0.5 per cent the total soluble nitrogen was about 60 per cent less and the ammonia nitrogen over 80 per cent less. The bacterial count with the higher concentration was only one-fourth of that found with the lower concentration. The ratio of ammonia nitrogen to total soluble nitrogen was lowered from 0.61 to 0.32.

Orthodichlorbenzene at a concentration of 0.2 per cent did not inhibit the growth of bacteria. The bacterial count was somewhat greater than with the control salt. In view of the absence of putrid odors and the low ratio of ammonia nitrogen to total soluble nitrogen it would appear that this material may act as an inhibitor of certain enzymes excreted by the bacteria and thus bring about a different kind of digestion.

Phenothiazine was not an effective preservative when added to the extent

of 0.4 per cent. The tendency for this material to permanently stain the skin brilliant red and blue under the influence of bacterial activity would seem to eliminate it from serious consideration.

The compound N–N′-dichloroazodicarbonamidine has bactericidal properties and a comparatively low reactivity with blood and tissue proteins as shown by Guiteras and Schmelkes[6]. According to Schmelkes and Horning[16] this compound can be considered as the oxidant of an oxidation-reduction system. The N–chloro group is dissociated much less than in other N–chloro compounds and, therefore, the chemical is quite stable and would tend to maintain the potential of the oxidant, or a relatively high potential. In studies on the physiology of halophilic bacteria[23] higher Eh values inhibited bacterial growth in media containing high concentrations of salt. Thus, it would appear that a compound of this type might be an effective preservative when used with salt.

As shown in Table II, N-N′-dichloroazodicarbonamidine-phosphate mixture (containing 3.17 per cent N-N′-dichloroazodicarbonamidine) did not increase the preservative efficiency of the control salt when added to the extent of 0.2 per cent. On the other hand, an equal amount of a mixture of the same organic compound with sodium bicarbonate (containing 33 per cent of N-N′-dichloroazodicarbonamidine) definitely increased the preservative efficiency of salt, as shown by the smaller values for total soluble nitrogen, ammonia nitrogen, and bacterial count. That the difference in the activity of the two mixtures is not due to the smaller amount of N-N′-dichloroazodicarbonamidine added with the phosphate mixture is brought out by the fact that the addition of 0.02 per cent of the bicarbonate mixture, a quantity containing roughly the same weight of the organic chemical as in ten times as much of phosphate mixture, resulted in a definite increase in the preservative efficiency of the control salt. The increases in total soluble nitrogen, ammonia nitrogen, and bacterial count were all less than with the phosphate mixture. The ratio of ammonia nitrogen to total soluble nitrogen was only 0.39 with the bicarbonate mixture as compared to 0.93 for the phosphate mixture. Increases in the concentration of the bicarbonate mixture up to 1 per cent did not increase the effectiveness of this organic chemical above that observed at 0.2 per cent.

N-N′-dichloroazodicarbonamidine imparts a yellow color to the salt but not to the skin. As long as there is a trace of this color present in the salt, the skin is practically perfectly preserved. As soon as the yellow color disappears, denoting a breakdown of the organic compound, bacterial growth develops. In these tests this occurred in from four and one-half to five weeks of incubation. The permanence of preservation with this compound is, therefore, still uncertain. The nontoxicity of the chemical to humans and other higher animals, its small apparent effect on the skin proteins, and its comparatively high preservative efficiency in low con-

centrations as long as the compound remains intact, are factors in its favor. Some pilot-scale tests with this material should be made to determine definitely just how stable it would be under practical working conditions.

The monohalogenated derivatives of the lower fatty acids have a biological action somewhat analogous to that of fluorides. A few tests were made to determine the effectiveness of a mixture of monochloracetic acid, sodium acetate, and potassium bromide as a preservative when added to salt. When this mixture alone or mixed with 0.1 per cent paranitrophenol was added to salt, the preservative efficiency of the salt was increased to an appreciable extent. Further studies should be made using the sodium salt of monochloro and monobromo acetic and propionic acids, both alone and in combination with other preservatives, since from these tests it would appear that they are highly effective in preserving salt-cured hides and skins.

Mixtures of 0.1 per cent paranitrophenol, sodium trichlorphenate, or sodium pentachlorphenate with 1 per cent of a sodium acetate-acetic acid buffer salt mixture did not give a preservative efficiency comparable to that obtained with a mixture of these same compounds with 1 per cent sodium silicofluoride. Thus, it appears that sodium silicofluoride has a preservative action greater than that which might be ascribed to its acid-buffering power[12].

Regarding the sodium salts of the chlorinated phenols, it should be pointed out that the phenolic derivatives themselves are but slightly soluble and, if their sodium salts are used with such acid salts as the sodium acetate-acetic acid buffer mixture, they probably would be precipitated as the insoluble phenolic derivatives. In spite of this, both sodium trichlorphenate and sodium pentachlorphenate, when used with the acetate buffer at pH 5.6, gave better preservation than when used with 1 per cent sodium carbonate. This is evident from both the appearance and the analytical data. With the acetic acid-sodium acetate buffer, sodium trichlorphenate is more active than sodium pentachlorphenate, as shown by lower values for the increases in total soluble nitrogen, ammonia nitrogen, and bacterial count. Thus, either alone or combined with acidic or basic salts, sodium trichlorphenate appears to be a more effective preservative than sodium pentachlorphenate.

The greater activity of the salts of the chlorinated phenols with the acid buffer mixture, as compared with that observed with the anhydrous sodium carbonate mixture, suggests that a strong alkaline reaction tends to destroy the bacteriostatic effectiveness of these compounds.

Cost is an important factor in the selection of any chemical for use with salt in curing hides and skins. With certain chemicals, however, it may not be safe to rule them out on the basis of cost alone since increased consumption might well result in a marked decrease in cost to the consumer. In small-scale laboratory tests of the kind reported herein exploratory studies with materials which at present are too costly to permit pilot-scale

studies can easily be made; the results will indicate the feasibility of further investigation.

Summary

A laboratory procedure for measuring the comparative preservative efficiency of chemicals that might be added to salt for better curing of hides and skins has been described. Some of the merits and limitations of this test are considered.

With this procedure the greatest preservative efficiency, with the chemicals and concentrations studied, was obtained with 1 per cent sodium silicofluoride combined with 0.1 per cent paranitrophenol, sodium trichlorphenate, or sodium pentachlorphenate.

One per cent sodium silicofluoride alone and 0.2 per cent sodium (2,4,5) trichlorphenate were also effective preservatives.

Although not quite so effective as the 2,4,5 derivative, sodium (2,4,6) trichlorphenate in a concentration of 0.2 per cent gave very good preservation.

Beta-naphthol in a concentration of 0.5 per cent, sodium pentachlorphenate at 0.5 per cent, sodium fluoride and sodium acid fluoride at 1.0 per cent, anhydrous sodium sulfite at 1.5 per cent, and N-N′-dichloroazodicarbonamidine at 0.07 per cent (when added with sodium bicarbonate) all gave good preservation. These compounds can therefore be classed as having enough promise to warrant further consideration.

Less effective, yet definitely adding to the preservative efficiency of salt, was a mixture of 1 per cent anhydrous sodium carbonate and 1 per cent naphthalene. Potassium perchlorate at 2 per cent, sodium perborate at 2 per cent, boric acid at 2 per cent, zinc chloride at 0.5 per cent, and colloidal zinc oxide at 1.3 per cent all brought about an appreciable increase in preservative efficiency.

Anhydrous sodium carbonate at a concentration of 3 per cent, acid sodium sulfate at 1.5 per cent, acid sodium sulfite at 1.5 per cent, anhydrous sodium sulfate at 40 per cent, phenothiazine at 0.4 per cent, and orthodichlorbenzene at 0.2 per cent did not appear to increase the preservative efficiency of salt.

The sodium salts of the chlorinated phenols were more effective as preservatives under acid conditions than in the presence of anhydrous sodium carbonate.

Mixtures of monochloracetic acid, sodium acetate and potassium bromide with salt gave good preservation; this suggests that the monohalogen derivatives of acetic and propionic acids may be useful as preservatives for use with salt in curing hides and skins.

References

1. Babakina, W. G., and K. S. Kutukowa, *Russ. Lederber.*, **5**, 102 (1934). Through W. Hausam, *Collegium*, **814**, 55 (1938).

2. Bergmann, M., *J.I.S.L.T.C.*, **16**, 2 (1932). Report of Pan-European Commission, No. 5. Raw Hides and Skins: Salting Problems.
3. Bergmann, M., and L. Seligsberger, *Collegium*, **751**, 814 (1932). Information on the Action of Soda Salt in Skin Conservation.
4. Das, M. B., B. B. Dhavale, and N. B. Pal, *J.I.S.L.T.C.*, **20**, No. 8, 381 (1936). The Curing of Hides with Different Mixtures of Sodium Sulfate and Sodium Chloride in Comparison with Khari Salt.
5. Frey, R. W., and L. S. Stuart, *J.A.L.C.A.*, **31**, 254 (1936). Pilot Scale Calfskin Curing with Treated Salt.
6. Guiteras, A. F., and F. C. Schmelkes, *J. Biol. Chem.*, **107**, No. 1, 235 (1934). The Comparative Action of Sodium Hypochlorite, Chloramine, and Azochloramid on Organic Substrates.
7. Grassman, W., and W. Hausam, *Ledertechn. Rdsch.*, **28**, No. 4, 25, and No. 5, 37 (1936). Report on Practical Salting Tests During the Year 1935.
8. Grassman, W., and W. Hausam, *Ledertechn. Rdsch.*, **30**, 57 (1938). Report on Practical Salting Tests During the Year 1937.
9. Guthrie, A., and M. S. Sastry, *J.I.S.L.T.C.*, **17**, 50 (1933). The Effect of Various Additions to Marine Salt for Use as a Cure for Hides and Skins.
10. Hausam, W., *Collegium*, **814**, 55 (1938). Progress Report Concerning the Conservation of Hides and Skins 1927-1937.
11. Kopenhoefer, R. M., and G. L. Somer, *J.A.L.C.A.*, **34**, 34 (1939). The Curing of Heavy Hides.
12. Marcovitch, S., *Univ. of Tenn. Agr. Exp. Sta. Bull.*, No. 139 (1928). Studies on Toxicity of Fluorine Compounds.
13. McLaughlin, G. D., and G. E. Rockwell, *J.A.L.C.A.*, **18**, 233 (1923). On the Bacteriology of the Curing of Animal Skin.
14. McLaughlin, G. D., I. H. Blank, and G. E. Rockwell, *J.A.L.C.A.*, **23**, 300 (1928). On the Re-Use of Salt in Curing Animal Skin.
15. Robertson, M. E., *J.I.S.L.T.C.*, **16**, 564 (1932). Red Heat—Its Cause and Prevention.
16. Schmelkes, F. C., and E. S. Horning, *J. of Bact.*, **29**, 323 (1935). Bactericidal Action of Azochloramid. (N-N′-Dichloroazodicarbonamidine)
17. Seidell, A., Solubilities of Inorganic and Organic Compounds, 2 vols., D. Van Nostrand Co., 1919.
18. Stuart, L. S., *J.A.L.C.A.*, **35**, 554 (1940). Studies on the Resalting of Salt-Cured Calfskins.
19. Stuart, L. S., and R. W. Frey, *J.A.L.C.A.*, **33**, 198 (1938). On the Spoilage of Stored Salted Calfskins.
20. Stuart, L. S., and R. W. Frey., *J.A.L.C.A.*, **36**, 114 (1941). Curing Skins with Salt Containing Sodium Carbonate or Sodium Sulfate.
21. Stuart, L. S., and R. W. Frey, *J.A.L.C.A.*, **29**, 630 (1934). Experiments with Salt Treated to Increase Its Hide Curing Ability.
22. Stuart, L. S., L. H. James, and R. W. Frey, *U. S. Dept. of Agr. Tech. Bull.*, 383 (1933). Microbiological Studies of Salt in Relation to the Reddening of Salted Hides.
23. Stuart, L. S., and L. H. James, *J. of Bact.*, **35**, 381 (1938). The Effect of Eh and Sodium Chloride Concentration on the Physiology of Halophilic Bacteria.
24. U. S. Patent No. 1,961,740. (V. Conquest).